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Incorporating anaerobic co-digestion of steam exploded or ammonia fiber expansion pretreated sugarcane residues with manure into a sugarcane-based bioenergy-livestock nexus

Thapelo Mokomele[a,b], Leonardo da Costa Sousa[b,c], Venkatesh Balan[b,d], Eugéne van Rensburg[a], Bruce E. Dale[b,c], Johann F. Görgens[a,*]

[a] Department of Process Engineering, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[b] Biomass Conversion Research Laboratory, Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI, USA
[c] Great Lakes Bioenergy Research Center (GLBRC), Michigan State University, East Lansing, MI, USA
[d] Department of Engineering Technology, Biotechnology Division, School of Technology, University of Houston, Houston, TX 77204, USA

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ABSTRACT

The co-digestion of pretreated sugarcane lignocelluloses with dairy cow manure (DCM) as a bioenergy production and waste management strategy, for intensive livestock farms located in sugarcane regions, was investigated. Ammonia fiber expansion (AFEX) increased the nitrogen content and accelerated the biodegradability of sugarcane bagasse (SCB) and cane leaf matter (CLM) through the cleavage of lignin carbohydrate crosslinks, resulting in the highest specific methane yields (292–299 L CH_4/kg VSadded), biogas methane content (57–59% v/v) and biodegradation rates, with or without co-digestion with DCM. To obtain comparable methane yields, untreated and steam exploded (StEx) SCB and CLM had to be co-digested with DCM, at mass ratios providing initial C/N ratios in the range of 18 to 35. Co-digestion with DCM improved the nutrient content of the solid digestates, providing digestates that could be used as biofertilizer to replace CLM that is removed from sugarcane fields during green harvesting.

1. Introduction

Current and future trends demonstrate that the increasing world population, dwindling arable land and increased demand for renewable energy present an opportunity to reconsider and redesign the manner in which land is used to meet future food, feed and bioenergy demands (Zilberman et al., 2013). With livestock production representing the largest anthropic use of global agricultural land, the adoption of intensified livestock farming practices (increased livestock per unit area) has been touted as a strategy for improving land use efficiency for food and bioenergy production, reducing deforestation, and enhancing economic returns for livestock farmers (Egeskog et al., 2011; Holm-Nielsen et al., 2009; Mazzetto et al., 2015).

Recent studies introduced a biorefinery concept whereby biomass pretreatment technologies are integrated into existing industrial sites (*e.g.* sugar/ethanol mills) for the production of conversion-ready biofuel feedstocks and highly digestible ruminant animal feeds from sugarcane crop residues (Dale et al., 2010; Egeskog et al., 2011). Our recent work has shown that pilot-scale ammonia fiber expansion (AFEX) and steam explosion (StEx) were effective treatments for simultaneously enhancing the *in-vitro* true digestibility and fungal enzyme degradability of sugarcane bagasse (SCB) and cane leaf matter (CLM) for ruminant feeding and/or ethanol production at industrially relevant conditions (Mokomele et al., 2018b). This concept is of particular interest to sugarcane and livestock dense regions such as Brazil, where an estimated 210 million cattle head are distributed over 167 million hectares of pasture land and more than 300 million tons of sugarcane residues are produced per annum (Dale, 2017). In South Africa, the major sugarcane producing regions also account for 54% of the national total cattle head, hence the potential use of StEx or AFEX treated sugarcane residues as animal feeds and the adoption of intensive feedstock production systems in these regions can increase land use efficiency (Carolan et al., 2007). However, intensive livestock farming systems are typically accompanied by the production of surplus animal manure, which represents a significant pollution risk with potential negative environmental impacts (Holm-Nielsen et al., 2009). Poor cattle manure

* Corresponding author.
E-mail addresses: mokomele@sun.ac.za (T. Mokomele), leo.sousa@egr.msu.edu (L. da Costa Sousa), vbalan@uh.edu (V. Balan), eugenevrb@sun.ac.za (E. van Rensburg), bdale@egr.msu.edu (B.E. Dale), jgorgens@sun.ac.za (J.F. Görgens).

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management practices, particularly for intensified livestock production systems, can significantly contribute to manure odor nuisance, manure disposal challenges, pollution of ground water, spreading of pathogens, and greenhouse gas emissions (Atandi and Rahman, 2012).

It is well known that anaerobic digestion (AD) of manure alone can lead to low biogas yields due to nutrient imbalance and ammonia toxicity to methanogens (Neshat et al., 2017; Xu et al., 2018). Anaerobic co-digestion of cow manure with agricultural residues can potentially harness the synergies between the two substrates by enhancing the digestion nutrient balance (*e.g.* C/N ratio, macro- and micro-nutrients), improving the digestion buffer capacity and potentially mitigating inhibition encountered during mono-digestion (Atandi and Rahman, 2012; Xin et al., 2018). Furthermore, co-digestion with regionally available residues produces farmer-owned renewable energy for on-farm use, increases the total biogas production capacity, reduces pathogen counts in the digestate, improves the fertilizer value of the digestate, and ultimately provides a more sustainable manure management strategy (Atandi and Rahman, 2012; Neves et al., 2009). Alternatively, depending on the proximity of the intensive animal farms to cellulosic biorefineries, the animal manure from these farms could be transported and co-digested with sugarcane residues in AD-based wastewater treatment plants that will form part of the water circuit of prospective cellulosic biorefineries.

Substrate hydrolysis is a one of the primary rate-limiting steps during AD of lignocellulosic biomass. Numerous pretreatment technologies (including StEx, ultrasound, NaOH, etc.) have been employed to unlock the recalcitrance of lignocellulosic biomass, thereby accelerating its anaerobic biodegradability for enhanced biogas production (Bolado-Rodríguez et al., 2016; Monlau et al., 2012; Risberg et al., 2013; Sambusiti et al., 2013). Among these pretreatments, De Paoli and co-workers (2011) reported the highest methane yields for SCB by using StEx pretreatment at 200 °C to achieve modest yields of 258 L CH_4/kg VS (De Paoli et al., 2011). In contrast, the combination of mechanical milling and 12% (w/w) NaOH pretreatment of CLM achieved methane yields of 291 L CH_4/kg VS, the highest methane yields reported in literature for CLM (Janke et al., 2017). However, the use of high alkaline concentrations limited the potential applicability of the digestates as fertilizer or soil application due to long-term salinization effects.

To date, there are no literature studies evaluating the methane production potential from AFEX treated lignocelluloses, neither in mono-digestion nor co-digestion with animal manure. Unlike most alkaline pretreatments, AFEX is demonstrated at pilot-scale with high ammonia recovery and has the unique characteristic of enhancing biomass biodegradability through the cleavage of ester-linked lignin carbohydrate complexes of monocots, particularly ferulates, diferulates and coumarates (Chundawat et al., 2011). In addition, AFEX fixes controlled amounts of degradable nitrogen onto the biomass resulting in C/N ratios in the range of 25–35 (Mokomele et al., 2018b). Coincidentally, these C/N ratios are within the optimum range recommended for efficient and stable AD (Njuguna et al., 2018).

Given the potential availability of SCB and CLM in sugarcane-producing areas, we deepened our consideration of the integrated biofuel and livestock production concept to incorporate the anaerobic co-digestion of dairy cow manure from intensified animal feeding systems with sugarcane residues for decentralized (farm-based) or centralized (cellulosic biorefinery-based) biogas production (Fig. 1). The aim of the present study was to experimentally assess the potential use of untreated, StEx- or AFEX-treated SCB and CLM as co-substrates with dairy cow manure (DCM) for high biogas production in batch anaerobic co-digestion systems. To achieve this, the impact of the two pretreatment technologies on the mono- and co-digestion of SCB and CLM was compared in terms of cumulative methane yield, methane content, biodegradation rate and total volatile fatty acid (VFA) production. Furthermore, an energy conversion assessment and solid digestate nutrient value was quantified for mono- or co-digestion substrates yielding specific methane yields greater than the mono-digestion of DCM, untreated SCB and untreated CLM. The results of this work provide insights into the incorporation of anaerobic co-digestion of sugarcane residues with DCM into the bioenergy-livestock production nexus for providing a more sustainable food-bioenergy-waste management approach for sugarcane and livestock dense regions.

2. Material and methods

2.1. Substrate, inoculum and cow manure

SCB and CLM were collected in the spring season of 2014 from two sugar mills located in Malelane (TSB Sugar, South Africa) and Mount Edgecombe (SASRI, South Africa) and prepared as previously described (Mokomele et al., 2018a). Inoculum was collected from an active farm-based anaerobic digestor (Durbanville, South Africa) that readily treats swine and cow manure under mesophilic conditions (~37 °C). The inoculum was degassed in a 30 L continuously stirred tank reactor (CSTR) under mesophilic conditions for five days to minimize endogenous methane production from any residual biodegradable organic material collected from the active digester. Fresh DCM was collected from lactating dairy cows consuming a typical total mixed ration diet at the Stellenbosch University Dairy Farm (Stellenbosch, South Africa), refrigerated at 4 °C, and used within 48 hrs. The total solids (TS) and volatile solids (VS) content of the inoculum and DCM are presented in Table 1.

2.2. Steam explosion and AFEX pretreatment

StEx pretreatment of SCB and CLM was carried out in an automated pilot scale unit equipped with a 19-L reaction vessel, 100-L discharge vessel and a 40-bar steam boiler (IAP GmBH, Graz, Austria). Further details on the StEx pretreatment protocol, experimental conditions and chemical composition adopted for these materials are found elsewhere (Mokomele et al., 2018a). The unwashed solid fraction after StEx was used in anaerobic biodegradability assays to evaluate the biomethane potential of StEx treated sugarcane residues. AFEX pretreatment was performed at pilot-scale using a pair of 450-L vertical packed bed reactors at MBI International (Lansing, MI, USA) (Mokomele et al., 2018b). Pretreatment conditions applied included an ammonia-to-biomass loading of 0.7 g NH_3/g DM, 0.6 g H_2O/g DM moisture content, non-uniform temperature range of 120–80 °C, and residence time of 60 min. Both SCB and CLM were pretreated at the same AFEX conditions.

2.3. Batch anaerobic digestion assays

Batch assays were conducted to evaluate the effect of StEx or AFEX pretreatment on the anaerobic biodegradability of SCB and CLM in mono-digestion and in co-digestion with DCM. Biomethane potential (BMP) assays were carried out in 100 mL serum bottles closed with a butyl rubber stoppers and sealed with aluminum crimps as previously reported (Holliger et al., 2016). Each assay was conducted at 6% total solids loading with an inoculum to substrate ratio (ISR) of 0.4 ($VS_{inoculum}/VS_{substrate}$). In preparation for BMP assays, the untreated and pretreated SCB and CLM samples were milled separately and passed through a 2-mm Wiley mill. The milled samples were added to the assay bottles with appropriate amounts of DCM, distilled water and inoculum to a final working volume of 70 mL. After inoculation, each assay bottle was sealed without pH adjustment, purged with N_2 gas for 2 min, and incubated at mesophilic conditions (37 ± 1 °C) for 55 days. Gas production was measured daily by volume displacement of a graduated syringe pierced through the butyl stopper, with the biogas composition quantified by gas chromatography (described below). The pH of each sample was measured before and after the BMP tests. For statistical inference, all assays were performed in triplicate.

To evaluate the effect of pretreatment and the effect of biomass-to-

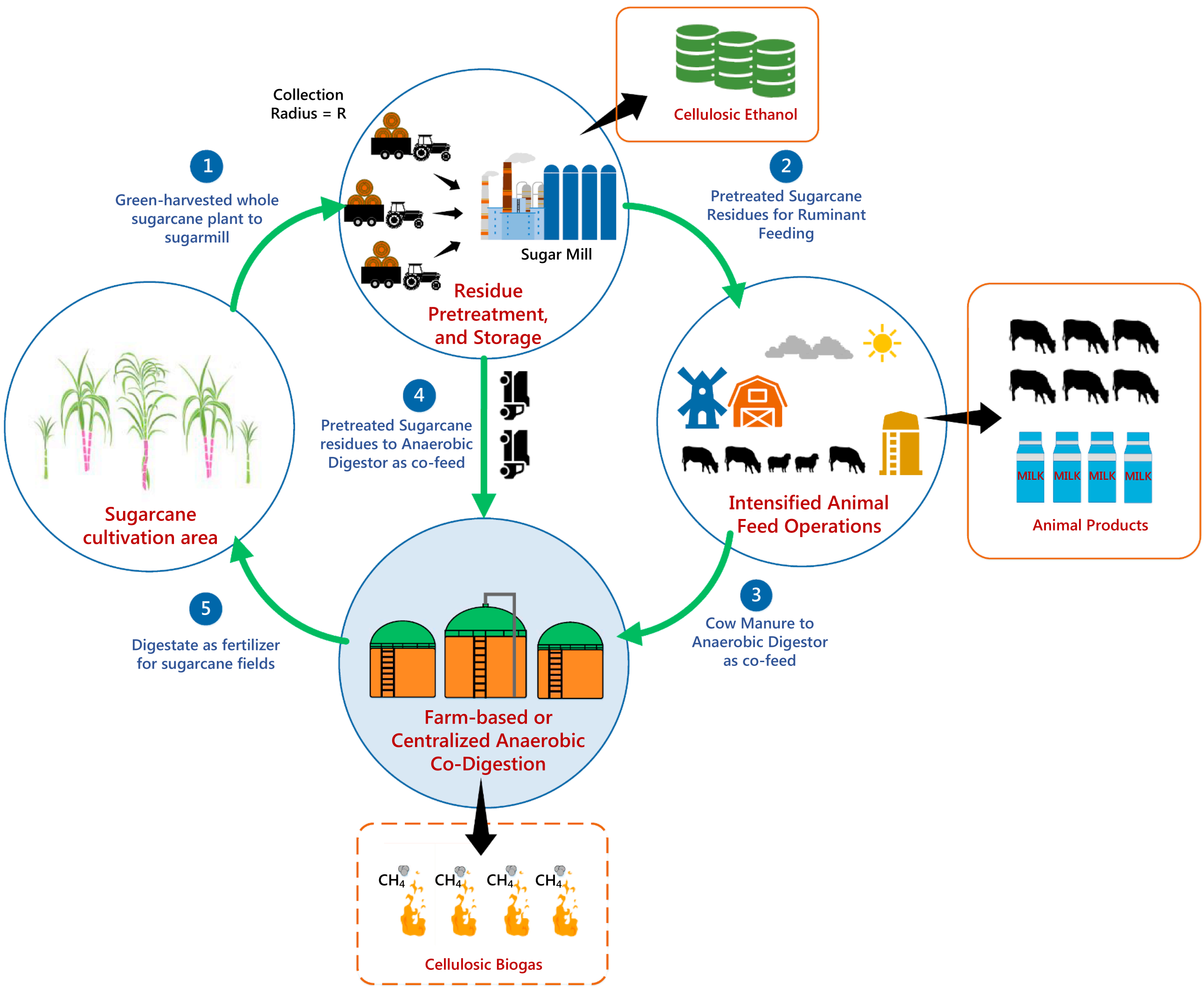

Fig. 1. Incorporating farm-based or centralized anaerobic co-digestion of sugarcane residues with livestock manure into integrated biofuel and livestock production systems for sugarcane and livestock dense regions.

Table 1
Total solids, volatile solids, chemical composition, macro-nutrient content, and calorific value of substrates used during anaerobic digestion assays.

Parameter	Sugarcane Bagasse			Cane Leaf Matter			Cattle Dairy Manure	Inoculum
	Untreated	AFEX	StEx	Untreated	AFEX	StEx		
% TS (% FM)	90.8 ± 0.8	90.8 ± 0.6	94.2 ± 0.5	93.3 ± 0.4	92.3 ± 0.8	92.9 ± 0.4	15.8 ± 1.1	2.7 ± 0.1
% VS (% TS)	96.1 ± 0.4	97.9 ± 0.3	96.1 ± 0.3	92.4 ± 0.3	91.9 ± 0.8	91.0 ± 1.1	83.7 ± 0.9	67.3 ± 1.5
pH	–	–	–	–	–	–	6.93 ±	7.61 ± 0.0
Cellulose (% TS)†	39.5 ± 0.4	39.5 ± 0.4	59.4 ± 0.5	37.5 ± 0.6	37.5 ± 0.6	55.3 ± 0.4	N/D	N/D
Arabinoxylan (% TS)†	26.4 ± 0.6	23.4 ± 0.6	7.1 ± 0.7	27.5 ± 0.7	25.5 ± 0.8	11.0 ± 0.4	N/D	N/D
Klason Lignin (% TS)†	19.3 ± 0.1	16.9 ± 0.1	29.5 ± 0.4	16.2 ± 0.8	14.4 ± 1.1	27.3 ± 0.3	N/D	N/D
% Carbon (C)†	45.8 ± 0.7	46.3 ± 0.8	48.0 ± 0.2	43.5 ± 0.2	43.9 ± 0.2	46.1 ± 0.3	42.0 ± 0.5	33.3 ± 0.6
% Nitrogen (N)	0.30 ± 0.0	1.46 ± 0.1	0.28 ± 0.0	0.41 ± 0.0	1.55 ± 0.1	0.38 ± 0.0	2.53 ± 0.1	2.39 ± 0.1
% Calcium (Ca)†	0.12 ± 0.0	0.11 ± 0.0	0.05 ± 0.0	0.33 ± 0.0	0.33 ± 0.0	0.22 ± 0.0	2.47 ± 0.1	3.63 ± 0.1
% Magnesium (Mg)†	0.06 ± 0.0	0.07 ± 0.0	0.06 ± 0.0	0.15 ± 0.0	0.15 ± 0.0	0.15 ± 0.0	0.64 ± 0.0	1.81 ± 0.0
% Phosphorus (P)†	0.06 ± 0.0	0.07 ± 0.0	0.01 ± 0.0	0.04 ± 0.0	0.03 ± 0.0	0.02 ± 0.0	0.47 ± 0.0	2.79 ± 0.1
% Potassium (K)†	0.13 ± 0.0	0.13 ± 0.0	0.01 ± 0.0	0.60 ± 0.1	0.59 ± 0.1	0.01 ± 0.0	0.70 ± 0.1	2.22 ± 0.2
% Sodium (Na)†	0.01 ± 0.0	0.01 ± 0.0	0.01 ± 0.0	0.04 ± 0.0	0.04 ± 0.0	0.04 ± 0.0	0.13 ± 0.0	1.48 ± 0.3
Sulphur (S)†	0.05 ± 0.0	0.06 ± 0.0	0.07 ± 0.0	0.27 ± 0.0	0.21 ± 0.0	0.04 ± 0.0	0.26 ± 0.0	0.95 ± 0.0
C/N ratio	153 ± 3	32 ± 1	172 ± 2	107 ± 1	28 ± 2	121 ± 1	17 ± 1	14 ± 1
HHV (MJ/kg)†	18.5 ± 0.1	19.1 ± 0.0	19.9 ± 0.0	17.7 ± 0.1	18.3 ± 0.1	18.9 ± 0.1	17.9 ± 0.0	13.8 ± 0.1
LHV (MJ/kg)†	17.1 ± 0.1	17.7 ± 0.0	18.6 ± 0.0	16.4 ± 0.1	17.0 ± 0.0	17.6 ± 0.0	16.6 ± 0.0	12.7 ± 0.0

N/D – not determined; TS – Total Solids; VS – Volatile solids; FM – fresh matter.
† % TS basis.

DCM mixing ratios, two sets of BMP assays were performed. The first set of BMP assays was performed to evaluate and compare the effect of StEx and AFEX pretreatment on the mono- and co-digestion methane yield, biogas methane content, biodegradation rate, and VFA production for both SCB and CLM. For this set of assays, co-digestion of untreated and pretreated SCB and CLM was performed at a fixed biomass-to-DCM ratio of 50:50 (VS basis), with mono-digestion of the DCM, untreated and pretreated SCB and CLM samples performed in parallel. A second set of BMP assays was performed to evaluate the effect of the ratio of biomass-to-DCM ratio on the specific methane production during anaerobic co-digestion. For these assays, untreated, StEx-treated and AFEX-treated CLM were used as the co-feeds at biomass-to-DCM mixture ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 (VS basis). For both sets of BMP assays, blank and positive control assays with no substrate and microcrystalline cellulose (Avicel PH-101) were included as reference assays to determine the background methane production and inoculum methanogenic activity, respectively.

2.4. Kinetic model analysis

A kinetic assessment of the batch BMPs was performed to compare the extent and rate of biodegradability of the various pretreated and co-digestion assays relative to untreated mono-digestion controls. The empirical Cone model was used to fit the measured specific methane yields for the domain t ≥ 0 days as described by Eq. (1).

$$\beta(t) = \frac{\beta_0}{1 + (kt)^{-n}} \tag{1}$$

In Eq. (1), β (L CH_4/kg VS_{added}) is the accumulated methane yield at time t; β_0 (L CH_4/kg VS_{added}) represents the maximal cumulative methane yield, k (day^{-1}) is the biodegradation rate constant, and n is the dimensionless Cone model shape constant (El-mashad, 2013).

2.5. Analytical techniques

Structural carbohydrates and Klason lignin contents of Lignocellulosic materials were determined according to National Renewable Energy Laboratory (NREL) protocols NREL/TP-510-42618 and NREL/TP -510-42620. The total carbon, nitrogen, hydrogen, and sulfur in all biomass samples were measured by elemental analysis conducted using a Vario EL Cube elemental analyser (Elementar GmbH, Germany). The macro-mineral content (Ca, Na, Mg, P,K, Fe) in biomass samples was quantified using a Thermo iCAP 6200 ICP-AES (Thermo Fischer Scientific, MA, USA). The biomass higher heating value (HHV) was measured using a bomb calorimeter (Cal2k Eco Calorimeter, RSA), which was previously calibrated with benzoic acid, in accordance with the ASTM standard D5865-11a. The lower heating value (LHV) was estimated from the measured HHV according to the European Standard (EN) 14918.

To qualitatively monitor functional group changes in pretreated lignocellulosic materials, Fourier Transform Infrared (FTIR) spectroscopic analysis of untreated, StEx- and AFEX-treated SCB and CLM samples was performed using a Thermo-Nicolet iS10 spectrometer operating in ATR mode with a diamond crystal. Spectra were obtained with an average of 64 scans for each sample at a resolution of 4 cm^{-1} in the range 650–4000 cm^{-1} using OMNIC® software.

Crystallinity of the cellulose fibers was evaluated using a D8 Advance X-Ray diffractometer equipped with a Lynxeye detector with its beam parallelized by a Goebel mirror (Bruker AXS Inc., Madison, USA). CuKα radiation was generated at an accelerating voltage of 40 kV voltage and an electric current of 40 mA. Scans were obtained from 2θ of 8.00° to 30.03° in increments of 0.02° and a scan rate of 5°/min. The crystallinity index (CrI) was calculated according to Eq. (2):

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \tag{2}$$

where I_{002} is the intensity of the diffraction from the 002-lattice plane at 2θ = 22.5°, and I_{am} is the intensity of diffraction at 2θ = 18.0°.

The biogas composition from BMP assays was determined using a gas chromatograph (CompactGC4.0, Global Analyzer Solutions, The Netherlands) equipped with two thermal conductivity detectors (TCD) for CO_2, CH_4, N_2, O_2, and H_2 quantification. Helium gas was used as the carrier gas at 5.0 mL/min and the operating temperatures of the injector, detector and column were 60 °C, 110 °C, and 65 °C, respectively. For analysis of VFAs, samples after AD were centrifuged at 10,000 rpm for 5 min before the supernatants were filtered through a 0.22 µm filter and subjected to HPLC quantification. The quantity of each VFA was measured using a Dionex UltiMate 3000 HPLC system equipped with UV detector (Thermo Fischer Scientific, UK). The column was a Bio-Rad Aminex HPX-87H ion exclusion column operating at 65 °C with 0.005 M H_2SO_4 as the mobile phase at a flowrate of 0.6 mL/min. The total VFA was calculated as the sum of the measured acetic acid, n-butyric acid, n-valeric acid, propanoic acid, and n-caproic acid.

2.6. Energy conversion assessment

An gross energy conversion assessment was carried out to evaluate the efficiency of AD in converting the heat of combustion energy in the inlet feedstocks into biogas equivalent energy using Eq. (3).

$$EC_{mixture}(\%) = \frac{V_{methane_{STP}} \times \rho_{methane_{STP}} \times LHV_{methane}}{\sum (m_i \times LHV_i)} \times 100 \tag{3}$$

where $EC_{mixture}$, $V_{methaneSTP}$, $\rho_{methaneSTP}$, and $LHV_{methane}$ represent the gross energy conversion factor for biogas relative to the inlet substrate mixture heat of combustion, the specific methane yield at standard temperature and pressure (STP: 273 K and 101.325 kPa), the methane density at STP (0.717 kg/m^3) and the net calorific value of methane at STP (50.4 MJ/kg). Similarly, m_i and LHV_i denote the mass and net calorific value of dry SCB, CLM, or DCM added to the BMP assays.

2.7. Statistical analysis

The statistical significance of experimental results was determined through a one-way analysis of variance (ANOVA) in combination with Tukey's HSD *post hoc* test for multiple comparisons (Minitab Inc., State College, PA, USA). The null hypothesis was accepted or rejected at a 95% confidence level ($P < 0.05$). Linear regression was performed in Minitab software to correlate the C/N ratio of various mono-and co-digestion experiments to the specific methane yields obtained from the BMP assays. The accuracy and significance of the regression equation was assessed using the coefficient of determination (R^2) and the regression model *P* value, respectively. Parameter estimation for the Cone model was performed with the least squares method using the Solver Function in Microsoft® Excel and the degree of fit was quantified using the Root Mean Square Error (RMSE) and Akaike's Information Criterion (AIC) as previously described (Motulsky and Christopoulos, 2003). To establish parameter estimation certainty, 95% confidence intervals of the Cone model parameters were computed using the Monte Carlo simulation approach in Microsoft® Excel as previously described (Hu et al., 2015).

3. Results and discussion

3.1. Substrate characteristics

The chemical composition, macro-nutrient content and gross calorific value of the DCM, inoculum, untreated, StEx- and AFEX-treated SCB and CLM are presented Table 1. AFEX-pretreatment significantly increased the nitrogen content of both SCB and CLM, resulting in substrates with C/N ratios of 32 and 28, respectively. During AFEX pretreatment, ammonolysis reactions cleave ether- and ester-linked lignin-

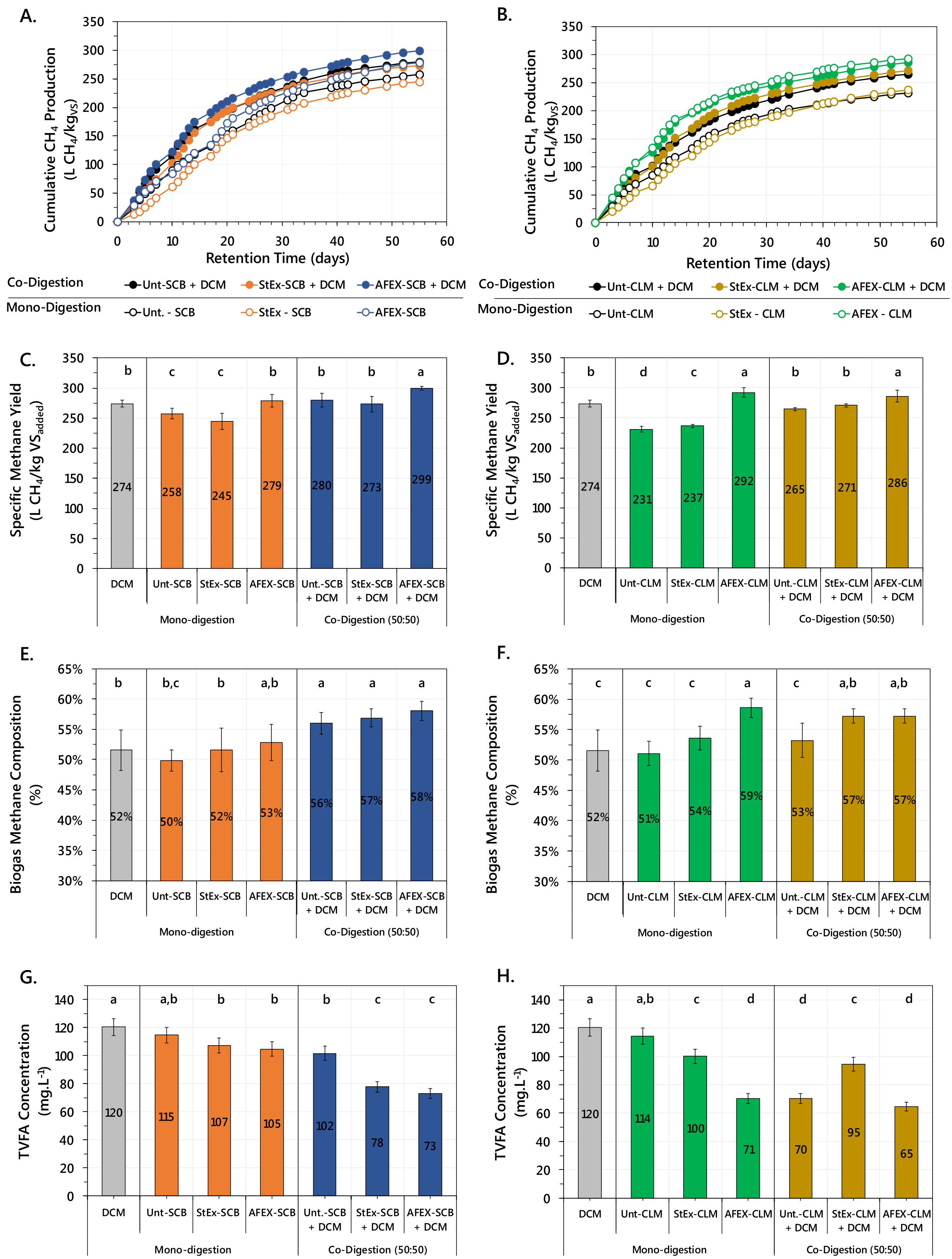

Fig. 2. Comparison of the cumulative methane production profiles (A,B), specific methane production (C,D), biogas methane content (E,F), and total VFA production (G,H) from mono-digestion and co-digestion (50:50) of untreated, AFEX-treated and StEx-treated SCB and CLM. Different alphabets above bar graph indicate significant differences as determined by one-way ANOVA with Tukey's post-hoc HSD test ($P < 0.05$).

carbohydrate crosslinks, resulting in the formation of nitrogenous compounds (predominantly acetamide and phenolic amides) that are chemically linked to the biomass (Chundawat et al., 2011). Furthermore, whereas nitrogenous Maillard reaction products have been recently quantified from AFEX treated SCB and CLM, these products were present in significantly lower quantities relative to the ammonolysis products (Mokomele et al., 2018b). Nonetheless, the AFEX derived nitrogenous compounds have been previously shown to be readily degradable by dairy cattle rumen microbes for bacterial protein synthesis, suggesting that these compounds may be valuable nitrogen sources for anaerobic digestion microbial communities (Blümmel et al., 2018; Mokomele et al., 2018b). In contrast, the high temperature StEx pretreatment resulted in the solubilization of 30–40% of the initial dry matter (mostly hemicelluloses and water/acid soluble extractives) into a liquor stream that was removed prior to AD (Mokomele et al., 2018a). Accordingly, the StEx-SCB and StEx-CLM substrates were enriched in cellulose (> 55%) and Klason lignin (> 27%) contents, and subsequently characterized by C/N ratios that were significantly higher than the untreated controls ($P < 0.05$). Moreover, unlike the untreated and AFEX-treated substrates, StEx-treated SCB and CLM demonstrated lower N, S, Ca, K and P contents, suggesting that these macro-nutrients were water soluble and, therefore, partially extracted into the liquid phase during StEx pretreatment.

3.2. Structural characterization of StEx/AFEX-treated SCB and CLM

To further investigate the structural modifications to SCB and CLM after StEx/AFEX pretreatment, comparison of the changes in the characteristic functional groups and crystallinity index relative to untreated controls were performed by ATR-FTIR and XRD analyses, respectively. In the fingerprint region (600–1800 cm^{-1}), FTIR spectra of AFEX-treated SCB and CLM demonstrated a significantly lower intensity of the 1240 cm^{-1} (ether linkages in hemicellulose and lignin), 1380 cm^{-1} (C—H deformation in hemicellulose and cellulose) and 1740 cm^{-1} (ester carbonyl C═O stretching) peaks relative to untreated controls, suggesting significant cleavage of ester linkages in lignin-hemicellulose complexes, acetyl groups and lignin side chains (Li et al., 2011). The appearance of the peak at 1664 cm^{-1} confirmed the formation of acetyl and phenolic amides, which are derived from the de-esterification of hemicellulose-lignin complexes by ammonolysis reactions (da Costa Sousa et al., 2016). Similarly, the reduction in bands at 1740 cm^{-1} and 1240 cm^{-1} in StEx treated SCB and CLM, suggest significant removal of hemicelluloses and/or cleavage of acetyl groups, consistent with the chemical composition presented in Table 1. In addition, the increase in peak intensity at 1440 cm^{-1} (H—O—C bending in hemicelluloses, lignin and cellulose), 1508 cm^{-1} (phenyl skeletal vibration of lignin), and 1600 cm^{-1} (C═C and C═O stretching in aromatic lignin) reflected enriched lignin content and potential presence of low molecular weight lignin fractions in StEx-treated SCB and CLM samples (Auxenfans et al., 2017). The increased intensity of bands at 1035 cm^{-1} (primary C—O/C—H groups stretching in cellulose) and 1160 cm^{-1} (secondary C—O/C—H group stretching in cellulose) relative to untreated SCB and CLM, may reflect the increased cellulose content in biomass due to hemicellulose removal (Zhang et al., 2011).

From the XRD spectra it was evident that StEx increased the CrI of SCB and CLM from 53% and 48% to 65% and 67% ($P < 0.05$), respectively, consistent with previous work with SO_2-impregnated StEx pretreatment of SCB (Corrales et al., 2012). It is well-documented that partial removal of amorphous cellulose and hemicelluloses by low pH pretreatments such as StEx results in a material that is enriched in crystalline cellulose and lignin. In contrast, slight increases in CrI were observed for AFEX-treated SCB (55%) and CLM (51%) relative to untreated controls ($P < 0.05$). This result agrees with previous work that suggested that pretreatment at high pH has less of an effect on cellulose crystallinity compared to pretreatment at lower pH values (Kumar et al., 2009).

3.3. Effect of StEx and AFEX pretreatment on methane yield and content after mono- and co-digestion

3.3.1. Mono-digestion trials

The effect of StEx and AFEX-pretreatment of SCB and CLM on the cumulative methane yield, methane content, and total VFA concentration after mono- or co-digestion is presented in Fig. 2. After a 55-day digestion period, the mono-digestion of untreated SCB, untreated CLM, and DCM produced specific methane yields of 258 ± 8.3 and 231 ± 4.6 L CH_4/kg of VS_{added}, respectively. The methane yields for these substrates were consistent and within the range of those reported in previous studies (De Paoli et al., 2011; Passos et al., 2017). The methane yield for DCM (274 ± 5.9 L CH_4/kg VS_{added}) was slightly higher than the literature-reported range of 130–255 L CH_4/kg VS_{added}, which can be attributed to the potential differences in the DCM chemical composition (influenced by the cows' diet), the near optimum C/N ratio of the DCM sample used in this work and the potential acclimation of the inoculum used in this work to swine and cow manure digestion (Chen et al., 2008; Zheng et al., 2015).

AFEX pretreatment of SCB and CLM generated the highest specific methane yields for anaerobic mono-digestion, enhancing the methane yields by 8% and 26% relative to the mono-digestion of untreated controls, respectively ($P < 0.05$). This can be explained by the structural changes that are attributed to AFEX-pretreatment, favorable substrate C/N ratio and lower Klason lignin content of the AFEX treated substrates relative to the untreated and StEx-treated substrates (Søndergaard et al., 2015). Further, we hypothesize that the additional nitrogen chemically linked to the biomass from AFEX pretreatment did not lead to excess ammonia accumulation, which would otherwise result in VFA accumulation, lowered digestion pH, and inhibition of the methanogenic community (Chen et al., 2016). Consequently, low total VFA concentrations (< 105 mg/L) were detected after AFEX-SCB and AFEX-CLM mono-digestion (Fig. 2G-H). These values were significantly lower than the literature-reported VFA inhibition concentration range of 1500–2000 mg/L (Neshat et al., 2017). However, time-based VFA, NH_4^+-N, and NH_3-N quantification is required to confirm the absence of ammonia inhibition. Nonetheless, the specific methane yield and biogas methane content for the mono-digestion of AFEX-CLM (292 ± 7.6 L CH_4/kg VS_{added} and 59 ± 1.6%, respectively) were statistically higher than that of DCM mono-digestion ($P < 0.05$), suggesting that the AFEX-CLM fibers were more biodegradable and/or the digestion nutrient balance was more suitable relative to DCM mono-digestion.

StEx pretreatment of SCB and CLM did not significantly improve or diminish the specific methane yields during anaerobic mono-digestion compared to untreated controls ($P > 0.05$). The extent of StEx-SCB and StEx-CLM anaerobic biodegradation might have been limited by the substrate characteristics, such as low biodegradable organic matter content, low digestion C/N ratio, high content of recalcitrant lignin, and the presence of toxic furan and phenolic moieties that are bound to the unwashed solids (Bolado-Rodríguez et al., 2016). Risberg et al., (2013) also reported insignificant differences for wheat straw steam-exploded at 210 °C and 10 min relative to untreated controls, citing the removal of biodegradable organic matter (predominantly hemicelluloses) and potential microbial community inhibition by pretreatment-derived compounds as limiting factors for StEx-treated substrate mono-digestion (Dale et al., 2016). Nevertheless, the cumulative methane yields for StEx-SCB and StEx-CLM mono-digestion achieved in this work (245 ± 13.2 and 237 ± 3.5 L CH_4/kg VS_{added}) were higher than those previously reported by Costa et al., (2014) and De Paoli et al., (2011) for hydrothermally pretreated SCB and steam-exploded CLM, respectively (Costa et al., 2014; De Paoli et al., 2011).

3.3.2. Co-digestion trials

The co-digestion of untreated, StEx-treated and AFEX-treated SCB and CLM with DCM at a mixture ratio of 50:50 (VS basis) significantly increased the specific methane yield for all the mixtures, except for the AFEX-CLM + DCM mixture, relative to the corresponding mono-digestion assays. Similar to the mono-digestion assays, the highest co-digestion methane yields were attained by the AFEX-treated substrates, with AFEX-SCB + DCM (299 ± 4.3 L CH_4/kg VS_{added}), enhancing methane yields by 16%, 7% and 9% relative to the mono-digestion of untreated-SCB, AFEX-SCB, and DCM, respectively ($P < 0.05$). With AFEX-treated SCB having high anaerobic biodegradability and a C/N ratio within the recommended optimum range, enhancement of the methane yield through co-digestion suggests that the DCM supplied the digesters with some essential macronutrients, micronutrients, and/or trace elements that may be required for maximizing the activity and synergy of the microbial population for degrading the AFEX-SCB + DCM mixture. For instance, DCM can provide supplementary cations such as Mg^{2+} Ca^{2+} and Fe^{2+} that are essential for the growth of methanogenic archaea and for stabilizing anaerobic digestion (Chen et al., 2008; Jackson-Moss et al., 1989). Accordingly, increased methane yield for the AFEX-SCB + DCM mixture beyond the additive contributions of each substrate indicated some synergistic effect caused by combining the two substrates (Søndergaard et al., 2015). For the nitrogen-limited untreated and StEx-treated SCB and CLM substrates, co-digestion with DCM potentially provides alkalinity for improving the digestion buffer capacity, nutrient balance and nitrogen to support microbial synthesis of amino acids, protein and nucleic acids (Neshat et al., 2017). As a result, co-digesting untreated and StEx-treated SCB and CLM with DCM increased methane yields by 8–15% relative to their mono-digestion counterparts ($P < 0.05$).

3.3.3. Kinetic analysis of methane production

Kinetic analysis and data modeling of methane production from mono-digestion and co-digestion of untreated, StEx-treated and AFEX-treated SCB and CLM were performed to evaluate the effect of pretreatment and co-digestion on the biodegradation rate constant (k) and the maximum methane yield (β_0). The estimated Cone model parameters are presented in Table 2 and the model prediction plots are available in Fig. 3.

Model simulations demonstrated that the Cone model adequately predicted the experimental mono- and co-digestion methane production profiles, as shown by the low RMSE and AIC and high R^2_{Adj} (> 0.995) for all the assays (El-mashad, 2013). The Cone model parameters were characterized by a narrow range of lower and upper 95% confidence interval limits, with the best-fitted parameters placed within this range, indicating high probability and certainty of the estimated model parameters (Hu et al., 2015; Motulsky and Christopoulos, 2003). As evidenced by the increased substrate biodegradation rate constants, the combination of biomass pretreatment and co-digestion with DCM significantly improved the substrate biodegradation rate relative to the mono-digestion for all the assays except for AFEX-CLM, suggesting that co-digestion was beneficial for improving the overall AD productivity and extent of digestion efficiency. The similar substrate biodegradation rate constants for the mono-digestion of AFEX-CLM and co-digestion of AFEX-CLM + DCM are explained by the similar C/N ratios of the two digestion mixtures (23 vs. 19), indicating that AFEX-CLM mono-digestion may already have sufficient fiber biodegradability, nutrient balance and buffer capacity to negate the benefits of DCM supplementation.

3.4. Methane production from co-digestion of untreated, StEx- and AFEX-treated CLM with DCM at different ratios

Cumulative methane yields from the mono- or co-digestion of untreated, StEx-treated and AFEX-treated CLM at biomass-to-DCM ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 (VS basis) are presented in Fig. 4. The variation of the AFEX-CLM:DCM co-digestion ratios did not have a statistically significant impact on the cumulative methane yields achieved relative to mono-digestion of AFEX-CLM ($P < 0.05$). This result may be due to the high biodegradability of AFEX-CLM and the narrow ranges of the C/N ratio (18–23) for all the AFEX-CLM + DCM mixtures. With instability of industrial AD plants being a major challenge, this result suggests that AFEX-treated CLM can be a valuable substrate for AD plants with non-uniform DCM supply. Apparently, AFEX-treated CLM can be digested at almost any mixture ratio without significantly reducing methane yields and biogas methane quality. In contrast, the co-digestion of untreated CLM + DCM at a biomass-to-DCM mixture ratio of 75:25 (C/N = 35) resulted in a significant increase in the cumulative methane yield relative to the mono-digestion of either DCM or untreated CLM ($P < 0.05$), suggesting some degree of synergy when mixing the two substrates at this ratio. Moreover, the methane yield of 292 ± 6.7 L CH_4/kg VS_{added} achieved with this substrate mixture was statistically comparable to AFEX-treated CLM in mono- and co-digestion with DCM ($P > 0.05$). For StEx-treated CLM, reducing the biomass-to-DCM ratio below 50:50 increased the digestion C/N ratio (> 50) and significantly reduced the cumulative methane yield (< 245 L CH_4/kg VS_{added}).

In support of the hypothesis that blending DCM with untreated or pretreated CLM significantly shifted the mixture C/N ratio and the corresponding cumulative specific methane yield, the biomass + DCM C/N ratios of the previously-mentioned mixtures were correlated with the specific methane yields obtained (Fig. 5). Within the wide range of C/N ratios considered in this study, a statistically significant negative

Table 2
Estimated Cone model kinetic parameters with the corresponding 95% parameter confidence intervals and degree of model fit.

Substrate	Estimated Cone Kinetic Parameters			Degree of Model fit		
	β_0 (L CH_4/kg VS_{added})	k (day^{-1})	n	R^2_{adj}	RMSE[†] (L CH_4/kg VS_{added})	AIC[‡]
DCM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	325 (315–338)	0.066 (0.061–0.070)	1.26 (1.19–1.32)	0.998	3.01	76.5
Untreated-SCB (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	367 (349–401)	0.040 (0.033–0.043)	1.23 (1.13–1.29)	0.997	4.36	100.2
StEx–SCB (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	287 (280–295)	0.050 (0.048–0.052)	1.77 (1.70–1.84)	0.998	3.32	82.8
AFEX-SCB (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	372 (345–407)	0.048 (0.042–0.052)	1.34 (1.21–1.47)	0.995	6.01	120.8
Untreated- CLM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	297 (281–315)	0.052 (0.047–0.058)	1.25 (1.15–1.34)	0.997	3.64	88.7
StEx–CLM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	296 (284–312)	0.048 (0.044–0.051)	1.46 (1.37–1.54)	0.998	3.32	82.7
AFEX-CLM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	336 (331–344)	0.078 (0.075–0.080)	1.29 (1.23–1.33)	0.998	3.09	78.1
Untreated-SCB + DCM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	342 (333–351)	0.063 (0.059–0.065)	1.24 (1.19–1.28)	0.999	2.41	62.4
StEx – SCB + DCM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	310 (303–318)	0.069 (0.066–0.072)	1.51 (1.45–1.59)	0.998	3.20	80.5
AFEX-SCB + DCM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	356 (346–367)	0.069 (0.064–0.072)	1.27 (1.22–1.33)	0.999	2.75	70.7
Untreated- CLM + DCM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	339 (326–355)	0.055 (0.050–0.059)	1.18 (1.14–1.25)	0.998	3.18	80.1
StEx – CLM + DCM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	314 (307–324)	0.065 (0.062–0.068)	1.44 (1.38–1.49)	0.998	3.35	83.4
AFEX-CLM + DCM (Lower $CI_{95\%}$ – Upper $CI_{95\%}$)	321 (314–334)	0.080 (0.074–0.083)	1.33 (1.25–1.39)	0.998	3.68	89.3

AFEX – Ammonia fiber expansion; StEx – Steam explosion; SCB – sugarcane bagasse; CLM; Cane leaf matter; DCM – Dairy Cow Manure.
[†] RMSE – root mean square error.
[‡] AIC – Akaike's Information Criterion.

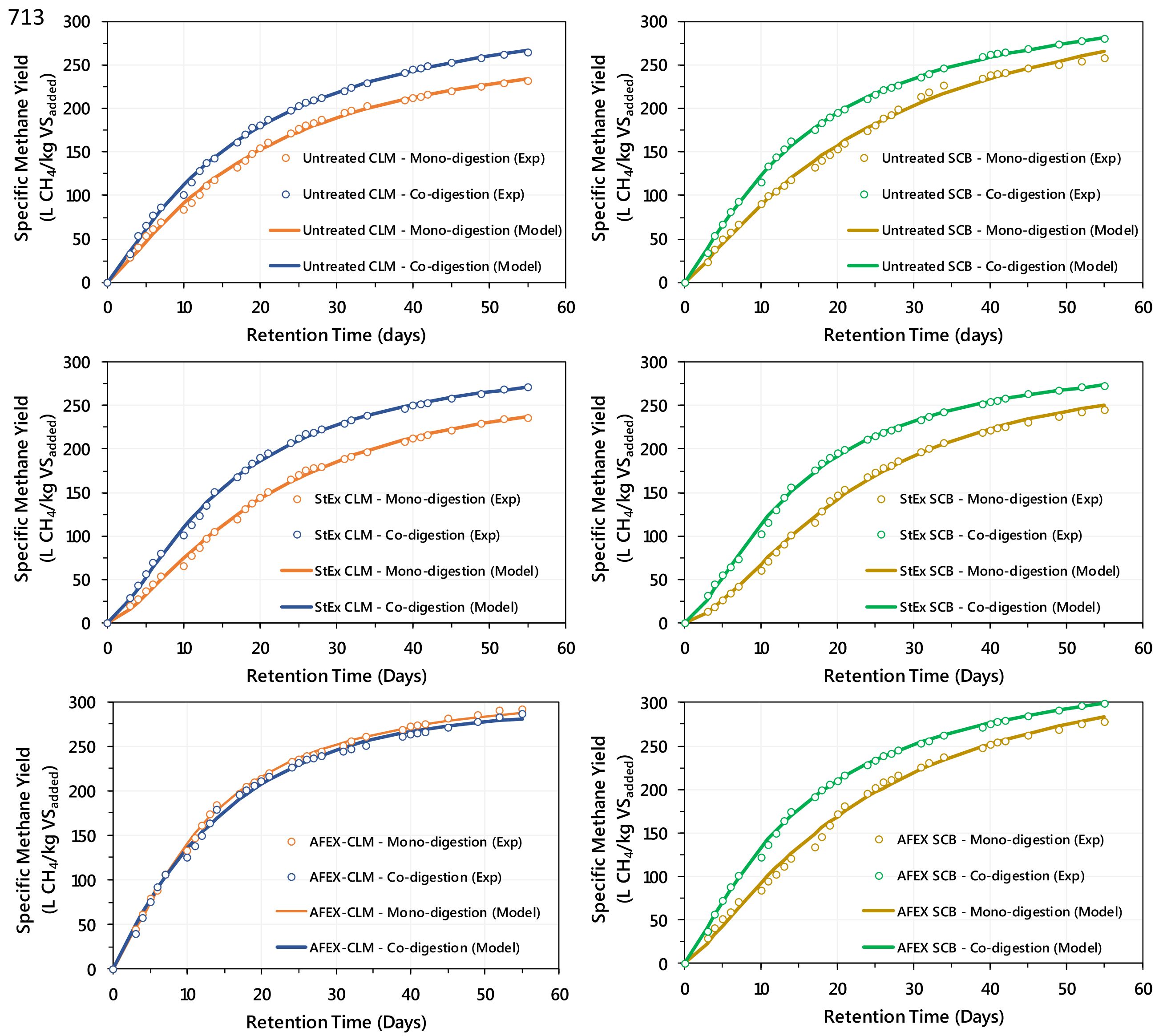

Fig. 3. Experimentally measured and Cone model predicted methane yield as a function of digestion time for mono- and co-digestion of untreated, AFEX-treated and StEx-treated SCB and CLM.

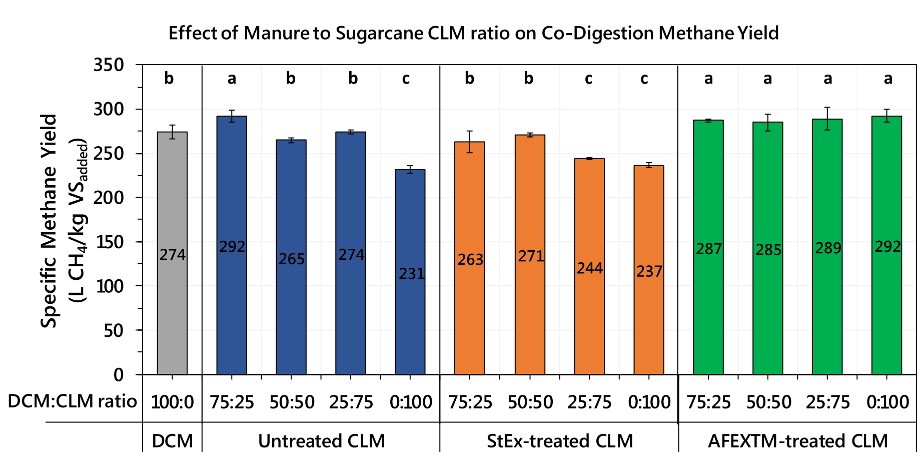

Fig. 4. Evaluating the effect of DCM-to-CLM ratio on the specific methane yield after anaerobic co-digestion for 55 days. Different alphabets above bar graph indicate significant difference as determined by one-way ANOVA with Tukey's post hoc HSD test ($P < 0.05$).

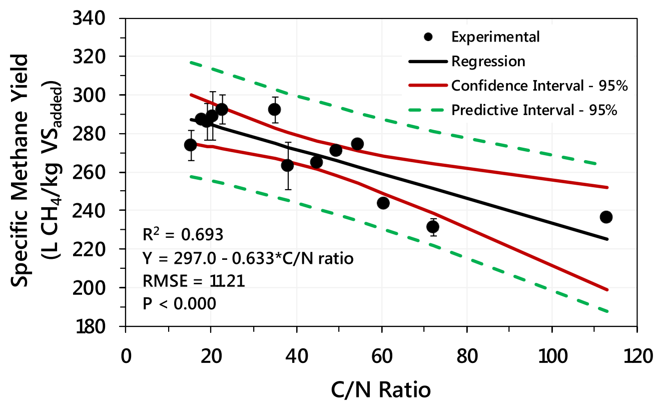

Fig. 5. Correlating the specific methane yields after anaerobic co-digestion to the inlet mixture C/N ratio.

linear correlation was found ($P < 0.0001$) between an increasing C/N ratio and the cumulative methane yield, with R^2 and RMSE values of 69.3% and 11.21 L CH_4/kg VS_{added}, respectively. Therefore, a linear correlation can explain 69.3% of the variation in the specific methane yield as a function of the C/N ratio within the wide C/N range of 15–113. This result also shows that the C/N ratio is not the only factor contributing to the specific methane yield and that factors such as fiber biodegradability, buffer capacity, micro- and trace element balance, and the dilution of toxic compounds are simultaneously influenced by various co-digestion ratios and, therefore, also significantly contribute to the cumulative methane yield variation (Chen et al., 2008). Nonetheless, based on the experimental data, the highest methane yields were achieved for mixture C/N ratios in the range of 18–35, comparable to the optimum range reported in literature (15–45) (Atandi and Rahman, 2012).

Janke and co-workers (2017b) combined mechanical milling and alkaline pretreatment (using 12 g NaOH/g DM) of CLM and reported the highest specific methane yields reported in literature for CLM (291 L CH_4/kg VS). However, although their AD process was not inhibited by the high Na^+ concentrations, it was also reported the high Na^+ concentrations in the digestate of the NaOH treated CLM could potentially limit the applicability of the digestate as fertilizer/soil conditioner due to its potential long-term soil salinization effect. In contrast, AFEX facilitated the methane yields that were greater than 290 L CH_4/kg VS (with or without co-digestion with cow manure), with high catalyst recovery (> 97%) and no negative impacts on the digestate quality. Similarly, the co-digestion of StEx treated SCB and CLM produced methane yields that were significantly higher than those reported for the mono-digestion of steam exploded sugarcane residues.

Practical considerations for selecting the preferred co-digestion ratio will depend on several factors including the relative amounts of sugarcane residues and DCM available to the AD plant, the size and number of domestic intensive, extensive and feedlot animal feeding systems, biomass/DCM storage and transportation logistics, seasonal availability of the untreated and/or pretreated sugarcane residues, and livestock farming interactions with domestic food production (Angelidaki and Ellegaard, 2003). Whereas AFEX-CLM offers process flexibility by maintaining high methane yields irrespective of the blending ratio with DCM, simply blending untreated CLM with DCM at the appropriate ratio may be a cheaper manure management solution for AD plants with adequate and consistent supply of DCM and sugarcane CLM. However, AD plants located in areas with limited DCM supply may experience reduced methane yields or even AD instability due to the oversupply of the untreated CLM/SCB in their digestion mixtures. Alternatively, for AD plants located near sugar mills supplying AFEX-treated CLM and SCB as feedstock to the cellulosic ethanol industry or as feed for intensified animal feed market, results from this work suggest that these AFEX-treated residues can perhaps be blended with untreated CLM/SCB and DCM to achieve appropriate C/N ratios to maximize cumulative methane yields and biogas methane content. Moreover, since AFEX facilitates easier crop residue pelletization, pelletized AFEX-treated sugarcane residues can be stored on-site, securing stable and high biodegradable biomass for farm-based or centralized AD plants located in areas with inconsistent year-round DCM supply (Sarks et al., 2016).

3.5. Energy conversion assessment and solid digestate fertilizer value

Anaerobic co-digestion of untreated CLM + DCM (25:75), AFEX-SCB + DCM (50:50), AFEX-CLM and AFEX-CLM + DCM (all mixtures) led to co-digestion C/N ratios that were within the range of 18–35 and subsequently resulted in methane yields that were statistically higher than the mono-digestion of untreated SCB, untreated CLM and DCM. An energy conversion assessment of these substrate mixtures was performed to estimate the ability of AD to convert the energy stored in the ingestates (non-digested substrate mixtures) into a methane-rich biogas stream (Table 3). In all cases of AFEX-pretreatment and/or co-digestion, the biogas energy recovery was in the range 50–53%, which was significantly higher than any of the mono-digestion cases ($P < 0.05$). These energy recoveries corresponded with volatile solids removal rates in the range of 56–60%, suggesting that a large portion of the ingestate energy remained in the recalcitrant solid digestate organic matter, which can be further valorized by conventional routes and used as soil amendments, biofertilizer, or dried and pelletized for thermochemical conversion in areas with domestic digestate oversupply (Monlau et al., 2015).

Table 3
Methane production, energy conversion efficiency and solid digestate fertilizer value for selected mono-digestion and co-digestion substrates.

Substrate	DCM	Untreated-SCB	Untreated-CLM	Untreated-CLM + DCM	AFEX-SCB + DCM	AFEX-CLM	AFEX-CLM + DCM
DCM/Biomass Ratio	100/0	0/100	0/100	25/75	50/50	0/100	50/50
Digestion C/N ratio	15	101	72	35	20	23	19
VS degraded in digestion (%)	51 ± 1.2% ^{C}	47 ± 2.1% ^{D}	42 ± 1.2% ^{E}	58 ± 1.3% ^{A,B}	60 ± 0.1% ^{A}	59 ± 0.7% ^{A}	56 ± 1.6% ^{B}
CH_4 Yield$_{STP}$ (Nm^3 CH_4/Mg VS_{added})	262 ± 10.7^{C}	246 ± 12.4 ^{D}	221 ± 6.1 ^{E}	286 ± 6.6 ^{A,B}	286 ± 3.2 ^{A}	280 ± 7.2 ^{A,B}	274 ± 9.2^{B}
Biogas CH_4 content (% v/v)	52 ± 3.4% ^{B}	50 ± 1.8% ^{B}	51 ± 2.0% ^{B}	55 ± 5.0% ^{A}	58 ± 1.6% ^{A}	59 ± 1.6% ^{A}	57 ± 1.2% ^{A}
Energy Conversion Efficiency (%)	47 ± 1.4% ^{C}	48 ± 1.7% ^{C}	41 ± 0.9% ^{D}	52 ± 0.5% ^{A,B}	53 ± 0.6% ^{A}	53 ± 1.5% ^{A,B}	50 ± 1.8% ^{B}
Solid digestate macro-nutrient value							
Total N (kg/Mg dry digestate)	33.6 ± 1.4	13.3 ± 0.3	14.4 ± 0.3	25.3 ± 1.2	24.4 ± 0.9	17.5 ± 0.7	26.1 ± 1.1
Total P (kg/Mg dry digestate)	23.1 ± 0.9	13.1 ± 0.5	15.3 ± 0.6	20.3 ± 0.8	21.2 ± 0.9	15.6 ± 0.5	19.1 ± 0.6
Total K (kg/Mg dry digestate)	13.0 ± 0.4	4.8 ± 0.1	12.5 ± 0.2	12.6 ± 0.3	11.5 ± 0.2	7.9 ± 0.1	11.1 ± 0.2
Total Ca (kg/Mg dry digestate)	66.0 ± 1.9	20.5 ± 0.6	26.0 ± 0.8	51.0 ± 1.5	44.0 ± 0.9	19.9 ± 0.4	45.6 ± 0.9
Total Mg (kg/Mg dry digestate)	20.9 ± 0.6	10.1 ± 0.3	10.5 ± 0.2	17.8 ± 0.5	17.3 ± 0.7	11.6 ± 0.5	16.2 ± 0.5
Total Na (kg/Mg dry digestate)	30.4 ± 1.2	21.9 ± 0.4	30.2 ± 0.5	30.3 ± 1.1	30.8 ± 1.1	18.3 ± 0.4	28.1 ± 0.9
Total Fe (kg/Mg dry digestate)	8.7 ± 0.2	3.0 ± 0.1	2.6 ± 0.1	5.3 ± 0.1	3.5 ± 0.1	1.6 ± 0.0	2.8 ± 0.1

STP – standard temperature pressure at 273 K and 101.325 kPa.

It is common practice to separate the AD digestate into liquid and solid digestate fractions for easier handling and storage. Macro-nutrient analysis of the solid digestate from the co-digestion assays showed that nitrogen, phosphate, and potassium (N-P-K) contents were more concentrated in the digestate relative to undigested SCB, CLM, and DCM (see Table 1). The NPK represented 5.7–6.8% of the total solids in the digestate, *i.e.* concentrated more than three-fold compared the raw SCB and CLM. An increased NPK content in the- solid digestate relative to the ingestate are typically attributed to the degradation of organic carbon to CH_4 and CO_2, microbial biomass, and the preservation and partial mineralization of N, P and K during AD (Tambone et al., 2010). Furthermore, the highest NPK, Mg, Ca, Na, S and Fe values were achieved for the co-digestion samples, implying that DCM supplementation adds additional essential minerals, which enhance the digestate fertilizer value.

For AD plants located near sugar mills and integrated to bioenergy-livestock systems, solid digestates may be used as either bedding for animals or combined with mineral-rich bottom ash from sugar mill cogeneration operations before being applied to the sugarcane fields as organic fertilizer or soil amendment to create a more sustainable biomass to food/bioenergy network (Eranki et al., 2011). A portion of the residual solids will be recalcitrant carbon and will, therefore, likely contribute to long-term carbon storage in the soil. This is an excellent example of bioenergy with carbon capture and storage (BECCS) system (Fajardy and Mac Dowell, 2018). Current sugarcane green harvesting techniques require that approximately 50% of the sugarcane CLM be left on the field to cover the soil in view of increasing nutrient recycling and soil organic matter, whilst minimizing temperature variation and water evaporation from the soil (Cantarella and Rossetto, 2014). The potential application of AD digestates with lower organic carbon and higher NPK as soil amendments and organic fertilizers in sugarcane fields may allow for more sugarcane CLM to be removed from the field after harvesting and allocated to bioenergy production, thereby improving bioenergy production yields per hectare of land. Alternatively, the AD digestates can be partial mineral fertilizer replacements, potentially minimizing fertilizer input costs for sugarcane growers (Dale et al., 2016; Walsh et al., 2012). For sugarcane and livestock dense regions, this strategy can potentially create a more sustainable food-bioenergy-waste management system. However, in-field tests may be necessary to understand the effects of increased CLM removal rates from the sugarcane fields and digestate application as partial mineral fertilizer substitute on the long-term sugarcane crop yields and productivity, soil fertility and environmental impacts.

4. Conclusions

The present study demonstrated that AFEX pretreatment significantly enhanced the specific methane yield, biogas methane content and biodegradation rate of the AD of SCB and CLM, with or without co-digestion with DCM. In contrast, nitrogen-limited untreated and StEx-treated SCB and CLM required blending with DCM to adjust the AD C/N ratio to 18–35 to achieve methane yields comparable to their AFEX counterparts. Co-digestion of sugarcane lignocelluloses within this C/N range facilitated the production of solid digestates with more concentrated NPK and lower organic matter relative to non-digested controls, suggesting that these digestates could partially replace CLM that is typically left on sugarcane fields.

Declaration of interests

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2018.10.049.

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